

**IN THE U.S. PATENT AND TRADEMARK OFFICE**

In re application of

Jean Pierre PLOUET et al.

Conf. 2413

Application No. 10/530,893

Group 1644

Filed February 27, 2006

Examiner M. Haddad

**TITLE**

NOVEL ENDOTHELIAL CELLS, ANTIBODIES DIRECTED AGAINST SAID  
CELLS AND USE THEREOF, IN PARTICULAR FOR SCREENING  
ANGIOGENESIS INHIBITING SUBSTANCES

**APPEAL BRIEF**

MAIL STOP APPEAL BRIEF-PATENTS  
Assistant Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

MAY IT PLEASE YOUR HONORS:

1. Real party in interest

The real party in interest in this appeal is:

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE, 3, RUE  
MICHEL-ANGE, F-75794 PARIS CEDEX 16, FRANCE.

2. Related appeals and interferences

None.

### 3. Status of claims

Claims 1-35 and 37-48 have been canceled. Claim 36 is pending in this application and has been finally rejected, from which this appeal is taken.

### 4. Status of amendments

No amendments have been filed subsequent to the final rejection mailed December 30, 2009. The claim at issue is the one set forth in the amendment filed September 21, 2009.

### 5. Summary of claimed subject matter

Independent claim 36: As is set forth in independent claim 36, the present invention pertains to a process for preparing a monoclonal antibody that acts against angiogenic endothelial cells (Page 8, lines 31-33). The antibody binds to a surface of the endothelial cells with an angiogenic phenotype (Page 8, lines 4-7). The antibody recognizes a unit present exclusively on those endothelial cells (Page 8, lines 8-11). The endothelial cells used to generate the antibodies are obtained by culturing endothelial cells removed from an aorta in a medium containing a supplement consisting essentially of oestradiol and VEGF (Page 7, lines 6-11). The endothelial cells form tubes in presence of growth factor VEGF in a collagen gel (Page 5, lines 3-4). The endothelial cells proliferate under action of VEGF (Page 5, line 5). The

endothelial cells are protected from apoptosis by VEGF (Page 5, line 6). The endothelial cells' expression of VEGFR-2 is increased 4-fold in comparison with cells with a non-angiogenic phenotype (Page 26, line 33 and Page 27, lines 1-2).

The method steps are (i):

immunizing an animal by injection of the endothelial cells with an angiogenic phenotype (Page 9, line 1); (ii) fusing myelomas of an animal and splenocytes of the animal in order to obtain hybridomas (Page 9, lines 2-3); (iii) preparing a culture of the hybridomas (Page 9, line 4); (iv) cloning of the hybridomas and secreting antibodies against endothelial cells with an angiogenic phenotype (Page 9, lines 5-6); and verifying that the antibodies inhibit said properties of said cells with an angiogenic phenotype (Page 9, lines 7-8).

#### 6. Grounds of rejection to be reviewed on appeal

The sole ground of rejection for review on appeal is whether claim 36 would have been obvious from SAITO et al. (Proceedings of the American Association of Cancer Research Annual Meeting, March 2002, Vol. 43, pp. 257) in view of CONCINA et al. (J. Vasc. Res. 2000 May-June; 37(3): 202-208) within the meaning of 35 U.S.C. §103(a).

## 7. Argument

### 7.0 Summary of Argument

The present invention makes antibodies that inhibit angiogenesis by immunizing an animal with endothelial cells that have been differentiated with only two necessary factors: oestradiol and VEGF. Moreover, these cells exhibit a 4-fold increase in expression of VEGFR-2, a factor promoting the production of antibodies of claim 36, which would have been unexpected in light of the applied art. The applied art teach away from claim 36, and no proper combination would have suggested either the method of that claim or the results thereby obtained.

### 7.1 Sole Ground - SAITO et al. in view of CONCINA et al.

The process for preparing monoclonal antibodies directed against angiogenic endothelial cells is generated by antibodies raised by injection of *"endothelial cells having an angiogenic phenotype"* that were in turn *"obtained by culturing endothelial cells removed from an aorta in a medium containing a supplement consisting essentially of oestradiol and VEGF."* (VEGF: Vascular Endothelial Growth Factor).

An additional aspect resides in that the endothelial cells' expression of VEGFR-2 is increased 4-fold in comparison with cells with a non-angiogenic phenotype (Claim 36, Page 26,

line 33 and Page 27, lines 1-2). This represent a result that is unexpected over the applied art.

SAITO et al. pertain to a method for producing monoclonal antibodies directed against epitopes expressed in the surface of tumor vasculature cells. For this purpose, SAITO et al. use Human Umbilical Vein Endothelial Cells (HUVEC) that are stimulated *in vitro* with proangiogenic factors, i.e., basic Fibroblast Growth Factor (bFGF), Vascular Endothelial Growth Factor (VEGF), and EEP, a growth factor obtained from new-born bovine brains added with murine Epidermal Growth Factor (EGF) and heparin. The cell treatment "activates" HUVEC cells, and they acquire an angiogenic phenotype.

SAITO et al. use a combination of growth factors that differs from the combination used in the present invention, i.e., oestradiol and VEGF only.

CONCINA et al. disclose that  $17\beta$ -Esteradiol (E2) (hereafter referred to as oestradiol) has mitogenic effects on *in vitro* endothelial cells derived from Fetal Bovine Aortic Endothelial Cells (FBAEC). This accounts for the stimulation of VEGF which will be secreted and will act in an autocrine way on the cell which has produced it. In a nutshell, CONCINA et al. describe exogenous oestradiol and endogenous VEGF whereas in the invention the cells are treated with exogenous oestradiol and exogenous VEGF.

CONCINA et al. also teach that oestradiol treatment of FBAEC cells stimulates the gene expression, and secretion, of VEGF, in a dose dependant manner. As mentioned in Figure 3, a dose of  $10^{-9}$  M of oestradiol allows the expression, and secretion, of 0.2 ng/ $10^6$  cells of VEGF and a dose of  $10^{-8}$  M of oestradiol allows the secretion of 0.3/ $10^6$  cells of VEGF.

Moreover, CONCINA et al. teach that it cannot be excluded that oestradiol might act on cells expressing VEGFR-2 and thus be incorporated in active angiogenesis.

The Office asserts that one of skill in the art would have immunized mice with oestradiol FBAEC treated cells, which express VEGF, to provide a method of producing antibodies reactive with tumor vasculature as taught by SAITO et al.

The Office argues that HUVEC taught by SAITO et al. and FBAEC taught by CONCINA et al. are both endothelial cells having an angiogenic phenotype, since they are both stimulated by angiogenic factors, and concludes that according to the *KSR v. Teleflex* decision (KSR, 550 U.S. at 416, 421), substituting a known element for another, to yield the known result, is obvious.

However, the combination of the teachings of SAITO et al. in view of CONCINA et al. teach away, since the combination of the teachings of these two documents would lead the skilled artisan in direction divergent from the path that

was taken by the applicant (see *In re Gurley*, 27 F.3d 551, 31 U.S.P.Q.2d 1130 (Fed. Cir. 1994)). This position is supported by the publications previously provided as evidence, which are attached to this paper and are discussed below.

The Office argues that HUVEC and FBAEC treated with angiogenic factors have to be considered both as endothelial cells with angiogenic phenotype.

At the filing date of the present application, the skilled person would know that many endothelial cells derived from veins, arteries, or arterioles can be used to provide, after stimulation with angiogenic phenotype, endothelial cells with an angiogenic phenotype.

Therefore, the posited document combination can be extended to other endothelial cells derived from veins, arteries, arterioles, placenta, capillary, retina, etc.

At the time the invention was made, the skilled person knew that endothelial cells used as model for studying *in vitro* angiogenesis are, for example, those set forth in Table 1 of VAILHE et al. (reproduced below), Laboratory Investigation, 2001, Vol. 81, No. 4, pp: 439-452, attached).

Table 1. In Vitro Models of Angiogenesis and Vasculogenesis

Cells	Mean time interval for the appearance of SCL	Incubation of immunogen <sup>a</sup>	Monoclonal	Spontaneous	Reference
HECIC, HECIC	3-6 h	Tumor and irradiated medium	Gelatin	2-0	Goldstein and Goldstein <sup>1</sup> , 1959
HECIC	4-6 h	S <sup>1</sup>	1-1- Fibrinogen, collagen dish	2-0	Masling et al., 1967
HAEC	1 wk	S	Cultured cells	2-0	Reznicek et al., 1980
HAEC	3-6 wk	S	Culture dish	2-0	Freese et al., 1981
HAEC and fetal calf	3 d	S	Amniotic membrane (Roussier substrate)	2-0	Martin and Williams, 1982
HAEC	3 d	1-1- Type IV and I collagen (microtiter)	Medium and filters	10-13	Martin and Williams, 1983
HAEC	3-10 d	S	Type I collagen gel	3-0	Schoen et al., 1983
HAEC	4 d	S	Cells suspended in type I collagen gel	3-0	Montesano et al., 1983
HAEC	3-12 d	S, OSB formation	Gelatin cast	3-0	Davies et al., 1984
MTF, ATC, HAEC	3-12 d	S	Fibrin Type I collagen gel	3-0	Montesano et al., 1985
HAEC, AEC, HEVFC	1 d	S	Fibrin	3-0	Chen et al., 1985
HAEC	10-16 d	S	Type I collagen gel	3-0	Montesano et al., 1986
HAEC	2-10	Pneumal water	Fibrin	3-0	Montesano et al., 1987
HAEC, HAEC, HAEC	1-10 d	S	Aluminate, collagen IV, Gelatin	2-0	Imhof and Folkner, 1988a
HAEC	1 wk	S <sup>1</sup>	Fibrin and Type I collagen gel	3-0	Nicastro and Ghera, 1989a and b
HAEC	10-16 d	S	Type I collagen gel	3-0	Vernon et al., 1992
HAEC	1 d	S	Cells embedded in fibrin or 1-1	3-0	Chazotte et al., 1995
HECIC, HEF	4-6 d	S	Microcarrier embedded in fibrin	3-0	Wong et al., 1996
CPAEC	2-7 d	S <sup>1</sup>	Fibrin	3-0	Hsieh and Hsueh, 1998a and b
HAEC, HEF	4-6 d	S	Microcarrier embedded in fibrin	3-0	Walsh et al., 1999
ISC	1-2 d	S, EB formation	Sensitized monolayer	3-0	Offner et al., 1980
HEVFC	7-21 d	S	Fibrin	3-0	Offner et al., 1984
HAEPF	2 wk	S	Collagen gel	3-0	Brown et al., 1990
HAEC	3-6 d	S <sup>1</sup>	Collagen gel	3-0	Acton et al., 1993
HAEC, HEVFC	3-9 d	S	Collagen gel	3-0	Vernon and Sapp, 1999
HAEC	1-2 d	S <sup>1</sup>	Type I collagen/fibrin	2-0	Kurtz and Angelier, 1999
				2-0	Pillitteri et al., 2000

[illegible]

<sup>2</sup> See references for accurate details.

Table 1 of VAILHE et al. teaches that all the mentioned cells can be used for *in vitro* angiogenesis and vasculogenesis. More precisely, Table 1 of VAILHE et al. teaches that BAEC (Bovine Aortic Endothelial Cells), from foetus or calf, are spontaneously able to induce morphogenesis when they are seeded on a plate without a cellular matrix component.

On the contrary, HUVEC (Human Umbilical Vein Endothelial Cells) and BREC (Bovine Retinal Endothelial Cells) cells are spontaneously able to induce morphogenesis when they are seeded on a plate coated with fibrin.

Thus, VAILHE et al. teach that BREC cells and HUVEC cells are the closest art, in terms of angiogenic potentialities, compared to FBAEC. Consequently, the skilled person would be motivated from the teachings of VAILHE et al.,



to replace HUVEC cells taught in the method by SAITO et al., by BREC cells instead of FBAEC cells.

At the time that the invention was made, one of skill in the art would have known that BREC cells stimulation by oestradiol induces VEGF gene expression in a dose dependant manner, and also induces VEGF protein expression. This is evidenced respectively in Figure 4 and Figure 5 of SUZUMA et al. (Investigative Ophtalmology & Visual Science, 1999, Vol 40, n. 9, pp: 2122-2129), reproduced below.

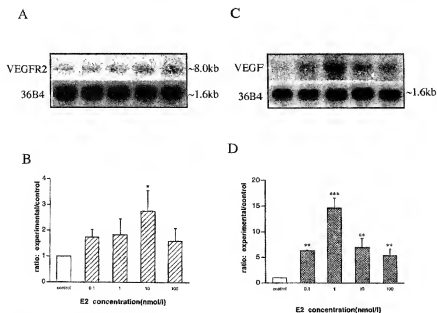
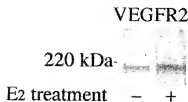


FIGURE 4. The effects of different doses of E<sub>2</sub> treatment on VEGFR2 (A, B) and VEGF (C, D) mRNA expression in BREC6. Subconfluent BREC6 were cultured with various concentrations of E<sub>2</sub> for 9 hours. Cells were collected, and 20 µl RNA extracts were analyzed by Northern blot analysis with VEGFR2 mRNA probe. (A) Results from representative Northern blot analysis are shown. (B) Densitometric analysis of the Northern blot analysis data. \*P < 0.05. (C, D) The effects of different doses of E<sub>2</sub> treatment on VEGF mRNA expression in BREC6. Subconfluent BREC6 were cultured with various concentrations of E<sub>2</sub> for 24 hours. Cells were collected, and 20 µl RNA extracts were analyzed by Northern blot analysis with VEGF mRNA probe. (C) Results from representative Northern blot analysis are shown. (D) Densitometric analysis of the Northern blot analysis data. \*\*\*P < 0.001, \*\*P < 0.01.

A



B

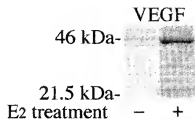


FIGURE 5. The effect of E<sub>2</sub> treatment on and VEGFR2 (A) and VEGF (B) protein expression in BREC cells. Subconfluent BREC cells were cultured with E<sub>2</sub>-depleted medium or 10-nM E<sub>2</sub>-replete medium. Cells were collected after 9 hours for VEGFR2 protein detection and after 24 hours for VEGF protein detection. Protein extracts were reacted with anti-VEGFR2 antibody or anti-VEGF antibody and immunoprecipitated with protein A-sepharose beads. Each sample was electrophoresed and analyzed using a densitometer.

Therefore, from the teachings of SUZUMA et al. and the teachings of VAILHE et al., the skilled person would be motivated to use the BREC of SUZUMA et al. instead of the FBAEC of CONCINA et al. to produce antibodies directed against tumor vasculature as disclosed in SAITO et al.

However, when substituting SAITO et al. cells by either SUZUMA et al. cells or CONCINA et al. cells, the skilled person would be led in direction divergent from the path that was taken by the applicant.

Indeed, claim 36 of the present invention sets forth that *"said endothelial cells having an angiogenic phenotype being obtained by culturing endothelial cells removed from an aorta in a medium consisting essentially of oestradiol and*

VEGF, said endothelial cells with an angiogenic phenotype being such that... their expression of VEGFR-2 is increased 4-fold in comparison with cells with a non-angiogenic phenotype".

This 4-fold increase in expression represents a result that is unexpected in light of the applied art. This result is thus analogous to the case where a many-fold improvement of activity over the prior art held sufficient to rebut *prima facie* obviousness based on close structural similarity. *In re Wiechert*, 370 F.2d 927, 152 USPQ 247 (CCPA 1967). Here, there is no structural similarity but rather a restriction to two factors: oestradiol and VEGF.

SUZUMA et al. teach that oestradiol treated BREC cells are able to express VEGF growth factor, but also VEGFR-2 receptor. Moreover, at an oestradiol dosage of  $10^{-8}$  M (i.e., 10 nM), the level of VEGFR-2 mRNA is increased  $2.4 \pm 0.3$  times compared to untreated BREC cells. This increase corresponds to the maximal increase at this dosage (see page 2126, second column, first paragraph).

In contrast to the teachings of SUZUMA et al., CONCINA et al. never mention that the oestradiol stimulation enhances VEGFR-2 gene expression, or the level of the aforesaid enhancement, if it exists. Without such information, one of skill in the art would be led to prefer SUZUMA et al.'s cells instead of CONCINA et al.'s cells.

Consequently, at the time the invention was made, one of ordinary skill, having a knowledge of all the prior art of relevance, would be seek to provide a method for producing antibodies specifically interacting with endothelial cells having angionenic phenotype, by using SUZUMA et al. BREC cells stimulated with oestradiol, and secreting VEGF. However, since one of the main features of the endothelial cells having an angiogenic phenotype used in this method is missing, the skilled person would be led in a direction divergent from the path that was taken by the applicant.

Indeed, since the cells used for the implementation of the method of SAITO et al. are different, the resulting monoclonal antibodies obtained by the method of SAITO et al. will be consequently different.

Attention should also be drawn to the fact that BREC cells are used in the application as filed, as a negative control. It is discussed in the Examples Section of the specification that FN cells have an increase of the VEGFR-2 mRNA expression compared to F/O cells, whereas the expression of VEGFR-2 mRNA is identical in BREC/O and BREC/V (Hutchings et al., 2002). The explanation is that Hutchings et al.'s BREC cells BREC/V have been stimulated, and thus activated, by the VEGF growth factor ONLY.

Indeed, the inventiveness of the present invention resides, in part, in that for the first time endothelial cells

with angiogenic phenotypes that have been stimulated by only two exogenous growth factor and hormones: VEGF and oestradiol. This particular growth factor and hormone stimulation confer to the cells specific new and inventive characteristics, allowing to obtain a new and inventive method for producing antibodies specifically directed against tumor vasculature.

Finally, if the skilled person has been motivated, for any reason, to combine the teachings of SAITO et al. and the teachings of CONCINA et al., the skilled artisan would obtain a method for producing monoclonal antibodies directed against endothelial cells having angiogenic phenotype, but the endothelial cells having angiogenic phenotype obtained would never have an expression of VEGFR-2 increased 4-fold in comparison with cells with a non-angiogenic phenotype.

Indeed, one of the aims of the present invention is the importance of the exogenous addition of VEGF in the cell culture medium, which has a significantly higher efficacy than the VEGF secreted in response to oestradiol treatment.

#### 7.1.1 Rebuttal to Office's Arguments

The Office Action of December 30, 2009 asserts that in contrast to Appellant's assertions of teaching away by the prior art because both CONCINA et al. and VAILHE et al. references indicate that the FBAEC as an *in vitro* model of angiogenesis and vasculogenesis (see Table 1, row 4 of VAILHE and Fig. 3 of CONCINA et al.), CONCINA et al. show FBAEC to be

sensitive to E2 stimulation in a dose-dependent manner; and that there is no discouragement nor skepticism in the prior art for the use of FBAEC in terms of angiogenic potentialities, particularly in light of the prior art teachings to provide the number of cells as a model for angiogenesis and vasculogenesis including FBAEC.

However, one of skill in the art would also be cognizant of the teachings of Figure 4 and Figure 5 of SUZUMA et al., discussed above, and be led to use BREO of SUZUMA et al. instead of FBAEC of CONCINA et al. to produce antibodies directed against tumor vasculature as disclosed in SAITO et al.

Referring to SUZUMA et al., the Office Action of December 30, 2009 asserts that While VAILHE et al. teach that BAEC (fetal and calf) can be used in *in vitro* models of angiogenesis and vasculogenesis (see Table 1, row 4), SUZUMA et al. uses the calf BAEC to study the effect of oestradiol on the induction of VEGF gene expression. CONCINA et al. use the fetal BAEC to also study the effect of E2 on VEGF content in conditioned medium of FBAEC. According to VAILHE et al. both fetal and calf BAEC can be used for *in vitro* models of angiogenesis and vasculogenesis, thus fetal and calf BAEC are interchangeable as an *in vitro* model for angiogenesis and vasculogenesis.

In rebuttal, it is respectfully submitted that these complex biochemistries are extremely unpredictable, and that there is no basis for the assumption that the foetal and calf BAEC are interchangeable in regards to the effect of E2 on VEGF content in a conditioned medium of FBAEC.

Referring to the 4-fold increase of expression, the Office Action of December 30, 2009 asserts that the expression of VEGFR-2 is increased 4-fold is material claim limitation, the statement of the intended result of supplementing oestradiol and VEGF does change VEGFR-2 expression or otherwise limit the claim. The Office Action further asserts that a person having ordinary skill in the art would have found it obvious to determine the optimum values of result-effective variables known in the art, and that the claimed angiogenic phenotype "expression of VEGFR-2 is increased 4-fold in comparison" of the FBAEC does not result in a manipulative difference in the method steps of the claims. The Office Action asserts that recitation of "expression of VEGFR-2 is increased 4-fold in comparison with cells with a non-angiogenic phenotype" is a statement of the intended results of the treatment of the FBAEC with oestradiol and VEGF, the combined reference teaching arrived to the use of oestradiol treatment which induces the release of VEGF.

However, the "expression of VEGFR-2 is increased 4-fold in comparison" is a limitation due patentable weight, and

not a result of optimization or intended result. That is, this term can be considered a functional limitation that is due patentable weight. *In re Swinehart*, 439 F.2d 210, 169 USPQ 226 (CCPA 1971). A functional limitation is often used in association with an element, ingredient, or step of a process to define a particular capability or purpose that is served by the recited element, ingredient or step. *Innova/Pure Water Inc. v. Safari Water Filtration Sys. Inc.*, 381 F.3d 1111, 1117-20, 72 USPQ2d 1001, 1006-08 (Fed. Cir. 2004).

Moreover, the 4-fold increase, even if claimed, is clearly an unexpected result in a very complicated field in which routine optimization is rare. The 4-fold increase is clearly set forth in the "Results" at pages 26 and 27 of the specification. Rebuttal evidence and arguments can be presented in the specification, *In re Soni*, 54 F.3d 746, 750, 34 USPQ2d 1684, 1687 (Fed. Cir. 1995), by counsel, *In re Chu*, 66 F.3d 292, 299, 36 USPQ2d 1089, 1094-95 (Fed. Cir. 1995). Examiners must consider comparative data in the specification which is intended to illustrate the claimed invention in reaching a conclusion with regard to the obviousness of the claims. *In re Margolis*, 785 F.2d 1029, 228 USPQ 940 (Fed. Cir. 1986).

The Office Action of December 30, 2009 finally asserts that the rejection was not made over SUZUMA et al. or VAILHE et al. but rather was made over CONCINA et al., and



that Appellant's arguments with respect to SUZUMA and VAILHE are irrelevant to the rejection of record.

However, SUZUMA et al. and VAILHE et al. were provided by the Appellant as evidence of the state of the art, to further demonstrate that the conventional art typified by CONCINA et al. would not lead one of skill (at the time the invention was made) to combine the references in a fashion to produce a claimed embodiment of the present invention.

Moreover, SUZUMA et al. and VAILHE et al. have been submitted for consideration in the IDS of September 21, 2009. Once the applicant has presented rebuttal evidence, Office personnel should reconsider any initial obviousness determination in view of the entire record. See, e.g., *In re Piasecki*, 745 F.2d 1468, 1472, 223 USPQ 785, 788 (Fed. Cir. 1984); *In re Eli Lilly & Co.*, 90 F.2d 943, 945, 14 USPQ2d 1741, 1743 (Fed. Cir. 1990).

Consequently, a person with ordinary skill would never obtain the present invention, as claimed, from a knowledge of the teachings of SAITO et al. and the teaching of CONCINA et al. in light of the art of record. A *prima facie* case of unpatentability has thus not been made. Moreover, any unpatentability that could be alleged is rebutted by the unexpected results.

Withdrawal of this rejection by the Board is accordingly respectfully requested.

8. Conclusion

The Appellant has demonstrated that the Examiner has failed to successfully allege that the rejected claim is *prima facie* unpatentable. It is clear that the inventive process for preparing monoclonal antibodies acting against angiogenic endothelial cells represents a truly inventive technology. For the reasons advanced above, it is respectfully submitted that the rejected claim in this application is allowable. Thus, favorable reconsideration and reversal of the rejection of the under 35 USC §103, by the Honorable Board of Patent Appeals and Interferences, are respectfully solicited.

Please charge the requisite Appeal Brief fee in the amount of \$540 to our credit card.

Respectfully submitted,

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REG/fb

May 18, 2010

9. Claims appendix

36. A process for preparing a monoclonal antibody directed against endothelial cells with an angiogenic phenotype that is capable of inhibiting angiogenesis,

said antibody being characterized by:

said antibody binds to a surface of the endothelial cells with an angiogenic phenotype, and

said antibody recognizes a unit present exclusively on the endothelial cells with an angiogenic phenotype,

said endothelial cells having an angiogenic phenotype being obtained by culturing endothelial cells removed from an aorta in a medium containing a supplement consisting essentially of oestradiol and VEGF,

said endothelial cells with an angiogenic phenotype being such that:

said endothelial cells form tubes in presence of growth factor VEGF in a collagen gel,

said endothelial cells proliferate under action of VEGF,

said endothelial cells are protected from apoptosis by VEGF, and

said endothelial cells' expression of VEGFR-2 is increased 4-fold in comparison with cells with a non-angiogenic phenotype,

comprising the steps of:

immunizing an animal by injection of said endothelial cells with an angiogenic phenotype;

fusing myelomas of an animal and splenocytes of said animal in order to obtain hybridomas;

preparing a culture of said hybridomas;

cloning of said hybridomas and secreting antibodies against endothelial cells with an angiogenic phenotype; and

verifying that said antibodies inhibit said properties of said cells with an angiogenic phenotype.

10. Evidence appendix

VAILHE et al., Laboratory Investigation, 2001, Vol. 81, No. 4, pp: 439-452.

SUZUMA et al., Investigative Ophtalmology & Visual Science, 1999, Vol 40, n. 9, pp: 2122-2129.

11. Related proceedings appendix

None.